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INDUCTION OF VOLATILE EMISSIONS IN MAIZE BY DIFFERENT LARVAL INSTARS OF *Spodoptera littoralis*

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Abstract—Maize plants under attack by caterpillars emit a specific blend of volatiles that is highly attractive to parasitic wasps. The release of these signals is induced by elicitors in the caterpillar regurgitant. Studies suggest that plants respond differently to different herbivore species and even to different herbivore stages, thus providing parasitoids and predators with specific signals. We tested if this is the case for different larval instars of the noctuid moth *Spodoptera littoralis* when they feed on maize plants. Cut maize plants were incubated in diluted regurgitant from second, third, or fifth instar caterpillars. There were no differences in total amount released after these treatments, but there were small differences in the release of the minor compounds phenethyl acetate and α-humulene. Regurgitant of all three instars contained the elicitor volicitin. To test the effect of actual feeding by the larvae, potted plants were infested with caterpillars of one of the three instars, and volatiles were collected the following day. The intensity of the emissions was correlated with the number of larvae feeding on a plant, and with the amount of damage inflicted, but was independent of the instar that caused the damage. We also used artificial damage to mimic the manner of feeding of each instar to test the importance of physical aspects of damages for the odor emission. The emission was highly variable, but no differences were found among the different types of damage. In olfactometer tests, *Microplitis rufiventris*, a parasitoid that can only successfully parasitize second and early third instar *S. littoralis*, did not differentiate among the odors of maize plants attacked by different instar larvae. The odor analyses as well

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as the parasitoid's responses indicate that maize odors induced by *S. littoralis* provide parasitoids with poor information on the larval developmental stage. We discuss the results in the context of variability and lack of specificity in odorous plant signals.

Key Words—larval instar, induced plant volatiles, specificity-reliability, 6-arm olfactometer, *Zea mays*, *Spodoptera littoralis*, *Microplitis rufiventris*.

INTRODUCTION

Plants subjected to feeding damage by insects respond with the release of characteristic blends of volatiles that attract parasitoids and predators (Dicke and Sabelis, 1988; Dicke et al., 1990; Turlings et al., 1990; Steinberg et al., 1992; McCall et al., 1993; Agelopoulos and Keller, 1994; Mattiacci et al., 1994; Röse et al., 1996; Du et al., 1998). These releases by attacked plants are triggered by elicitors in oral secretions of the herbivores (Dicke et al., 1993; Turlings et al., 1993a; Mattiacci et al., 1994). In the regurgitant of the caterpillar *Pieris brassicae*, the main elicitor was identified as the enzyme β-glucosidase (Mattiacci et al., 1994). Alborn et al. (1997, 2000) identified a nonprotein elicitor from the regurgitant of *Spodoptera exigua* (Lepidoptera: Noctuidae), *N*-(17-hydroxylinolenoyl)-L-glutamine, named volicitin. In maize plants, volicitin triggers a response similar to that triggered by *S. exigua* feeding. The induced odor is composed mainly of terpenoids and is highly attractive to the braconid parasitoids *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Microplitis croceipes* (Hymenoptera: Braconidae) (Alborn et al., 1997; Turlings et al., 2000).

Interestingly, *M. croceipes* cannot parasitize *S. exigua* larvae, but is nevertheless highly attracted to maize odors induced by this nonhost (McCall et al., 1993; Turlings et al., 1993a). This potential limitation to the reliability of herbivore-induced plant signals has been discussed by Vet and Dicke (1992). They argue that the large quantities of the plant-provided cues make them highly detectable, but they may be poor indicators of herbivore identity. However, some recent studies suggest that plant signals can be herbivore specific. De Moraes et al. (1998) demonstrate that the parasitoid *Cardiochiles (Toxoneuron) nigriceps* (Hymenoptera: Braconidae) can distinguish odors from plants damaged by its specific host from odors induced by a nonhost. Differences in the attractiveness to the wasps were mainly ascribed to quantitative differences in a few compounds released by the plants. Guerrieri et al. (1999) showed that different aphid species elicit different emissions in bean plants and that the aphid parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae: Aphidiinae), can use these to distinguish plants infested by its host, *Acyrthosiphon pisum* (Homoptera: Aphididae), from those infested by a nonhost, *Aphis fabae* (Homoptera: Aphididae). These studies suggest that herbivores can induce specific signals in plants that give information on the identity of potential hosts for parasitoids, but in some other systems this appears not to be the

case (McCall et al., 1993; Turlings et al., 1993a). Another intriguing example of specificity is that reported by Takabayashi et al. (1995) who showed that the parasitic wasp *Cotesia kariyai* (Hymenoptera: Braconidae) can differentiate between maize plants under attack by young instars and late instars of *Pseudaletia separata* (Lepidoptera: Noctuidae). In contrast, in the cabbage system, composed of cabbage plants–*Pieris brassicae* (Lepidoptera: Pieridae)–*Cotesia glomerata* (Hymenoptera: Braconidae), the parasitoid does not discriminate between plants infested by first and fifth instars (Mattiacci and Dicke, 1995). This was surprising because chemical analyses of collected cabbage odors suggested that the host instars differed in the odor they induced in the plant. The specificity of plant responses seems to differ for different systems, and the reliability of the information provided by the chemical signals varies accordingly.

In the current study, we tested if, in the tritrophic system comprising maize plants, *S. littoralis* (Boisd.) caterpillars, and the endoparasitoid *M. rufiventris* (Kok.),the induced odor differs with the developmental stage of the herbivore on the plant and whether such differences are detected and used by the wasp, which can only attack early instars. For this purpose, volatiles emitted by maize plants that were subjected to various treatments were collected and analyzed. Plants were incubated in the regurgitant of second, third, and fifth instars. They were also subjected to actual feeding damage by these three instars. Finally, artificial damage was used to mimic the damage caused by the instars. We also tested if *M. rufiventris* distinguished among the odors from plants attacked by different instars. The amount of damage was the main factor that determined the intensity of the odor emissions and their attractiveness. No major differences were found in the identities and relatives ratios of the various compounds. The results indicate that in this system odor emitted by the maize plant provides no or poor information on herbivore instar.

METHODS AND MATERIALS

Plants. Maize of the variety Delprim was used. Seeds were sown in individual plastic pots (360 ml, 10 cm diam. 7 cm high) filled with fertilized soil (Coop, Switzerland). Plants were grown in a climate chamber (type 10'US/+5 DU-PI, Weiss Umwelttechnik, Switzerland) at 23°C, 60% relative humidity, 45,000 lux, and under a 16L:8D light regime. Plants 9–10 days old were used for all experiments, at which age they carry 3–4 leaves.

Insects. S. littoralis larvae and eggs were received weekly from Syngenta (Stein, Switzerland). Batches of eggs were placed on moist filter paper in a Petri dish. Newly hatched larvae were put on maize leaves (var. Delprim), in transparent plastic boxes (13.5 × 15 × 5 cm). Larvae of second, third, and fifth instars were used. Regurgitant was collected as described by Turlings et al. (1993a).

A colony of the parasitoid *M. rufiventris* was started with cocoons provided by Dr. E. Hegazi (University of Alexandria, Alexandria, Egypt). The colony was maintained on *S. littoralis* larvae fed with artificial diet. At emergence, adults were sexed and kept in plastic cages (30 × 30 × 30 cm, Bugdorm I, MegaView Science Education Service Co, Ltd, Taiwan) under laboratory conditions (25 ± 3°C, 40% relative humidity). Insects were provided with honey, and cages were sprayed with water daily to compensate for relatively dry lab conditions.

Collection and Analysis of Induced Odor. Two systems were used to collect induced odors from plants. The first system was an all glass push–pull odor collection system modified from Turlings et al. (1991). It consists of three cylindrical Pyrex glass pieces. The first tube (14 cm) contains a glass frit, which ensures laminar airflow into the second tube. It ends with a 6-cm male ground-glass joint that is connected to a female counterpart of the second tube (29 × 7 cm). This second tube ends in a female ground glass joint (3 cm) that fits the inlet of the third tube, which tapers down and ends in a glass screw fitting. An open screw cap with a Teflon-covered rubber ferrule (6 mm ID) connects a collection trap at its upwind end on the third glass tube, while the downwind end of the filter is connected with plastic tubing to flowmeters (5-channel Adjustable Vacuum Flow Volatile Collection System, model VCS-5ASP-MAN, Analytical Research Systems, Gainesville, Florida, USA), which are connected to a vacuum pump. Humidified and purified air is pushed and pulled through the glass tubes at a rate of 600 ml/min. Collection traps were made as described by Heath and Manukian (1994). Before each collection, filters were rinsed with 500 μl of pentane, followed by 500 μl of methylene chloride.

The second system was designed to collect from growing plants (Turlings et al., 1998). The aerial part of a plant was placed in a vertical cylinder (9.5 × 54 cm), while the pot was placed outside, separated from the rest of the plant by a Teflon disk (Turlings et al., 1998). Purified and humidified air was pushed into each cylinder at a rate of 1 liter/min. For collections, air was pulled through a trap held at the base of the cylinder, at a rate of 0.8 liters/min, while the rest of the air vented through the hole in the bottom, thus preventing outside, impure air from entering. The automated part of the collection system (Analytical Research Systems) controlled the flow. The climate chamber (CMP4030, Conviron, Winnipeg, Canada) in which the collection cylinders were housed was kept at 17.5°C. Due to irradiation heat, the temperature inside the cylinders was 23 ± 3°C. During the light cycle, light intensity was about 20,000 lm/m^2.

After each collection, filters were extracted with 150 μl of methylene chloride (Lichrosolv, Merck), and 200 ng of *n*-octane and nonyl acetate (Sigma) in 10 μl methylene chloride were added to the samples as internal standards.

Analyses were done with an Hewlett Packard HP 6890 series GC equipped with an automated on-column injection system (HP G1513 A) and a flame ionization detector. A 3-μl aliquot was injected onto an apolar EC-1 capillary column

(30 m × 0.25 mm ID, 0.25-μm film thickness, Alltech Associates) preceded by a deactivated retention gap (10 m × 0.25 mm ID, Connex) and a deactivated precolumn (30 cm × 0.530 mm ID, Connex). Helium (24 cm/sec) was used as carrier gas. Following injection, the column temperature was maintained at 50°C for 3 min, increased to 230°C at 8°C/ min, and held at 230°C for 9.5 min. The detector signal was processed with HP GC Chemstation software. Compound identities had been determined in previous studies (Turlings et al., 1998; Gouinguené et al., 2001).

Effect of Regurgitant on Induction of Odor. Oral secretions of second, third, and fifth instars were tested. Three plants were used. The cut stem of each seedling was incubated in a solution of regurgitant (10% in distilled water) during one night (14–15 hr). Controls were incubated in distilled water only. Before collection, the part of the stem that had been submerged in the solution was cut off, and the fresh cut was wrapped in a piece of moist cotton to avoid desiccation. Three plants that were treated with the same regurgitant solution were placed together in a glass tube for odor collection. The experiment was replicated 13 times. Total amounts as well as the composition of the induced odor blend emitted by the plants treated with different regurgitants were compared.

Compositions of the three instars were compared to detect any variation in elicitor quality or quantity. Regurgitant samples were collected as described above. To denature enzymes and to eliminate bacterial degradation, each sample of oral secretion was diluted with an equal amount of acetonitrile immediately after collection. Samples were centrifuged at 14,000 rpm (Eppendorf Centrifuge 5415) to remove solids, and the supernatant was filtered through 0.45- and 0.22-μm sterilizing membranes (Millex-HV and Millex-GV, Millipore Bedford, Massachusetts, USA). For quantitative analyses, 5 μl of the internal standard *N*-palmitoleoyl-L-glutamine solution (1 μg/μl) in CH_3CN/H_2O (8:2 v/v) were added to each sample (50 μl) as an internal standard. Ten microliters of each sample were analyzed by HPLC with UV detection at 200 nm (constaMetric 4100 pump, SpectroMonitor 3200 detector, Spectra System AS 3500 autosampler, Thermo Separation Products, Riviera Beach, Florida, USA). A reverse-phase column (YMC-Pack ODS-AMQ, 250 × 4.6 mm ID, YMC, Kyoto, Japan) was eluted (1 ml/min) with a solvent gradient of 20–95% CH_3CN (High Purity Solvent, Burdick & Jackson, Muskegon, Mississipi, USA) containing 0.8% acetic acid (Aldrich, Milwaukee, Wisconsin, USA), in water (Milli-Q UV PLUS system, Millipore) containing 0.5% acetic acid, over 40 min, and then returned to the initial conditions at 45 min. Column temperature was maintained at 60°C. The detector response to the internal standard was used to calculate the amounts of *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), *N*-(17-hydroxylinoleoyl)glutamine, 17-hydroxylinolenic acid, 17-hydroxylinoleic acid, *N*-linolenoyl-L-glutamine, *N*-linoleoyl glutamine, linolenic acid, and linoleic acid. These were isolated and identified earlier in beet armyworm (*S. exigua*) oral secretions (Alborn et al., 2000),

and the structures confirmed by using methods described in Mori et al. (2001).

Effect of Feeding by Second, Third, and Fifth Instars. To test if larvae of different instars induced different volatiles, we collected odor from plants attacked by different numbers of larvae. For second instars, either 1, 5, 10, 25, 50, or 70 larvae were placed on one plant. For third instars, the numbers were 1, 5, 10, 20, or 50 larvae. These numbers were chosen to obtain comparable amounts of damage. For the fifth instars, the densities were lower. Due to the size and feeding rate of the larvae, only 1, 2, 3, 4, 5, or 10 larvae were put on a plant. A cellulose bag (Celloclair, Liestal, Switzerland) was placed over each plant to prevent escape of caterpillars. Larvae were placed on the plants in the evening and were allowed to feed for 15 hr. They were removed just before an odor collection. After collection, the amount of damage on plants was estimated visually and expressed as the percentage of leaf surface removed.

Effect of Different Types of Damage. The damage caused by the different instars of *S. littoralis* is different. Young instars graze the surface of the leaf, creating "windows" and leaving most of the veins intact, while late instars remove all parts of a leaf. Intermediate instars combine both types of damage: they partly graze on the leaves and remove small parts of tissue. To determine if the different types of damage are correlated with differences in odor emissions, we mimicked the caterpillar damage. To mimic second-instar damage, the surface of the leaf was removed with a razor blade in order to leave the veins intact. In each case, 2 cm^2 were damaged per leaf. To mimic third-instar damage, the same manipulation was done, and the leaves were cut in some places. Again, 2 cm^2 were damaged per leaf. For fifth-instar damage, 10 holes were punched per leaf. Holes were 4 mm in diameter, which corresponds to a surface of 5 cm^2 per leaf, but the damaged area remaining was considerably less. For all treatments, 10 μl of caterpillar regurgitant were applied on the damaged area. Two plants were used per treatment. Plants were treated in the evening (18:00 hr), and kept in the dark until collection (09:30 hr the next morning). This was replicated nine times.

Does M. rufiventris Distinguish among Plants Fed on by Different Instars of S. littoralis? Choice tests were conducted in a six-arm olfactometer. It consists of an airtight three-part system of glass chambers interconnected by Teflon tubes. On the bottom, six glass chambers each contain a small growing plant, and purified and humidified air enters these chambers. Air passes over the plants and is pushed to the upper part of the system, where it enters the arms of the olfactometer. Half the air is pulled out at the upper part of the chamber through volatile collection traps (Heath and Manukian, 1994), which allows for trapping part of the volatiles during each bioassay. The upper part consists of a six-arm glass star, and each arm is connected with a Teflon tube to an odor source chamber from the bottom part. Thus, the air from each chamber passes through one arm to the central chamber of

the star. This central chamber is a glass cylinder that extends into the middle part of the system where the wasps are released. At the bottom, just below the release point, the remaining air is pulled out of the system. Wasps that are released in the chamber readily walk up in the direction of a light source that is placed above the olfactometer. Once they have reached this upper part they can make a "choice" for an odor by walking into one of the arms.

As odor sources, three undamaged plants were alternated with plants fed on by each instar of *S. littoralis* caterpillars in the six bottom chambers. The maximum emission of odor occurred at about 60% damage on a plant, independent of the instar. This amount of damage was correlated to a corresponding number of larvae for each instar tested. Based on this, plants were fed on by either 60 second instars, 30 third instars, or 3 fifth instars for 15 hr. Odor emissions from plants fed on by the different instars were collected for 3 hr. Four groups of six female parasitoids were tested each day. One group of six was naive females, while the other trials were conducted with six female wasps that had had an oviposition experience with plants that had been damaged by either second, third, or fifth instars overnight. During such experiences, female parasitoids learn to associate the odor they encounter with the presence of hosts and become more responsive to this odor (Lewis and Tumlinson, 1988; Turlings et al., 1993b; Vet et al., 1995). If the odors differ among the three instars, this is reflected in the responses of the different experience groups. The position of the plants remained the same on a particular day to avoid an effect of odor contamination in the arms, but the position of treated plants and control plants was changed between days of experiments. The day before each replicate, the system was thoroughly washed, rinsed with solvents, and the glass parts placed in an oven at 250°C for several hours. Experiments were conducted on 10 different days (60 wasps per experience type).

Statistical Analyses. The total amount of induced odor released was compared using one-way ANOVA. Data were ln-transformed to comply with ANOVA assumptions. The Student-Newman-Keuls test was used as the *post hoc* test for multiple comparison. Comparison of the amounts of the 20 dominant compounds was done using a multivariate ANOVA. The Dunnett T3 *post hoc* test was used for multiple comparisons.

Quadratic regressions on ln-transformed data were performed to test for the effect of the quantity of damage on the release of induced volatiles. The analysis was done for each instar separately. Confidence intervals of equation coefficients were calculated and compared. For comparison of the odor blends induced by the three instars, data were grouped in five damage classes. Multivariate analysis of variance was performed to compare the effect of instar and of the class of damage on the release of six dominant compounds. Dunnett T3 *post hoc* test was used for multiple comparison.

Chi-square analysis was performed to test for differences among proportions of wasps that choose the different odors they were offered.

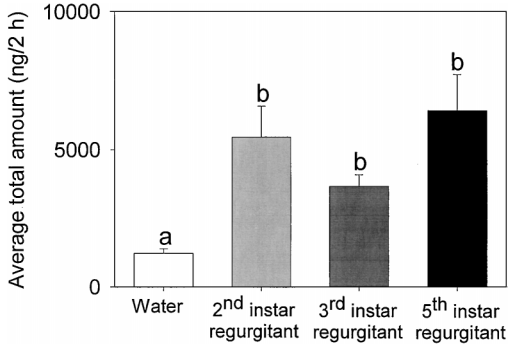

FIG. 1. Average total amount + SE of induced odor emitted by maize seedlings after incubation in a solution with regurgitant of a specific instar. Incubation in water was used as control. Different letters above each bar indicate differences among treatments according to Student-Newman-Keuls *post hoc* test after one-way ANOVA ($F = 16.138$; $P < 0.001$)

RESULTS

Effect of Regurgitant on Odor Induction. No significant differences were found in the total amount of induced volatiles emitted by maize plants incubated in regurgitant solutions from second, third, and fifth instars of *S. littoralis* (Figure 1). As expected, plants incubated only in water released significantly less compared to plants incubated in regurgitant solutions (Figure 1).

Composition of the odor blend induced by the different regurgitants was not significantly different, except for the compounds phenethyl acetate and α-humulene, which were released in higher relative proportions by plants incubated in fifth-instar regurgitant solution as compared to other treatments (Figure 2).

Analysis of the regurgitant of the three instars tested showed that all contained volicitin. For all instars, linolenic acid dominated the composition of the regurgitant. The ratios of the different compounds present varied among the three instars (Figure 3). Only one sample of second-instar regurgitant was available, so no statistical analysis was performed, but any possible difference among the three instars is not dramatic, and all instars produced volicitin.

Effect of Feeding by Second, Third, and Fifth Instars on Induced Emissions. The percentage of damage done to maize plants was correlated with the number of larvae feeding. Not surprisingly, the relationship between the quantity of damage and the number of larvae differed for the different instars (Figure 4). For each instar, the amount of volatiles released and the amount of damage inflicted closely fit a quadratic relationship (Figure 4). The maximum emission of induced odor occurred in plants from which about 60% of the surface had been removed (Figure 4). The confidence intervals for each coefficient of the three equations overlapped

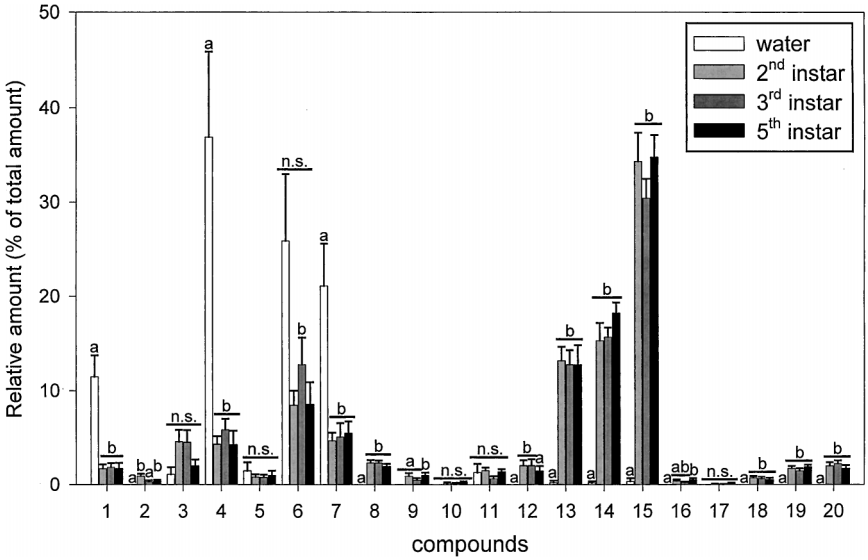

FIG. 2. Average relative amount + SE of the main compounds released by maize seedlings after incubation in a solution with regurgitant of a specific larval instar. Incubation in water was used as control. Different letters above bars indicate significant differences among treatments for the different compounds according to Student-Newman-Keuls test. Peak identities: (1) (*Z*)-3-hexenal, (2) (*E*)-2-hexenal, (3) (*Z*)-3-hexen-1-ol, (4) (*E*)-2-hexen-1-ol, (5) β-myrcene, (6) (*Z*)-3-hexen-1-yl acetate, (7) linalool, (8) (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (9) phenethyl acetate, (10) indole, (11) geranyl acetate (12) unknown, (13) β-caryophyllene, (14) (*E*)-α-bergamotene, (15) (*E*)-β-farnesene, (16) α-humulene, (17) unknown sesquiterpene, (18) β-bisabolene + (*E*, *E*)-α-farnesene, (19) β-sesquiphellandrene, (20) (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

almost completely, which means that the amount of induced volatiles released after caterpillar feeding did not differ among the three instars.

The quality of the odor blend also depended on the amount of damage done by each instar (Figure 5). There were no obvious differences among the three instars tested. Two sesquiterpenes, α-bergamotene and (*E*)-β-farnesene, were dominant in the odor blend for all instars. Figure 5 illustrates the ratios of the main compounds in the induced blend for five classes of damage and for the three instars. Multivariate analysis of variance indicated that the instar had no effect on the amount of the different induced volatiles ($F = 1.164$, $P = 0.279$), while the classes of damage had an effect ($F = 1.676$, $P = 0.004$), and the intercept between the instar and the classes was not significant ($F = 0.896$, $P = 0.755$). Among the compounds, linalool, indole, β-caryophyllene, α-bergamotene, and (*E*)-β-farnesene were released in significantly different amounts among the different

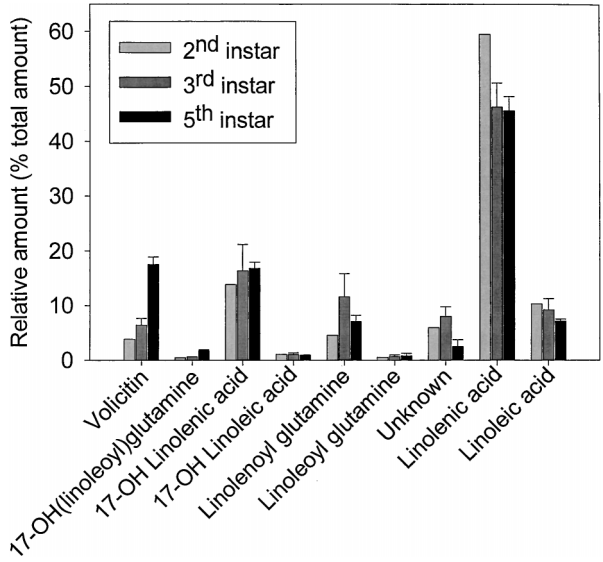

FIG. 3. Relative amount (mean + SE) of the different components present in the regurgitant of 2nd, 3rd, and 5th instars of *S. littoralis*.

classes of damage. The relative amount of linalool decreased when the amount of damage increased, except between 40 and 60% of damage where plants damaged by second instars emitted a large amount of linalool. The proportion of indole increased in the odor blend with the amount of damage. The proportion of β-caryophyllene in the induced odor blend increased slightly with the amount of damage (Figure 5). About 20% of the induced odor was composed of α-bergamotene, which represented a slightly higher proportion when 40–60 % of a plant was damaged. The same trend was observed for (E)-β-farnesene, which was the main substance in the induced blend (about 40%, Figure 5).

Effect of Different Types of Damage. Total amounts of induced volatiles for the three types of damage were not significantly different from each other, but were different from the undamaged plant (Figure 6). Multivariate analysis of variance indicated that the type of damage also had no effect on the composition of the blend (Figure 7, $F = 1.728$, $P = 0.923$).

Does M. rufiventris Distinguish among Plants Fed on by Different Instars? Female parasitoids significantly preferred the odor of plants that were fed on by larvae to undamaged plants; on average 87% of the females made a choice for the odor of damaged plants. When females had no previous experience or had experience with odors of plants fed on by third or fifth instars, no preferences were found in their choices (Figure 8A, C, D). Surprisingly, female wasps

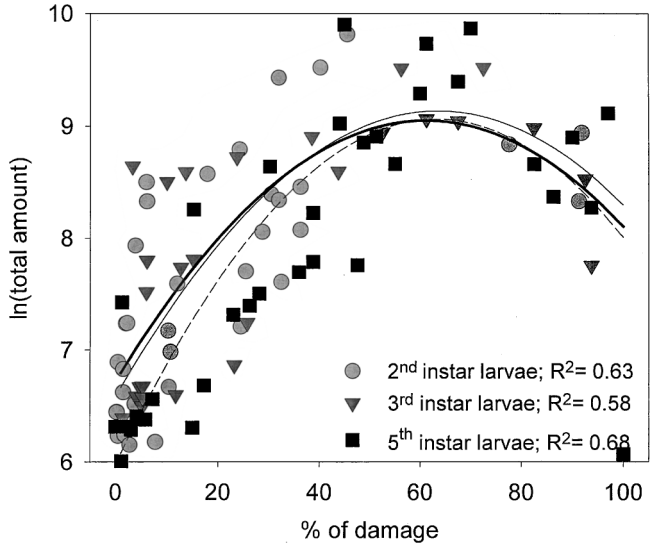

FIG. 4. Total amount of induced volatiles emitted by second, third, and fifth instars (ln-transformed data) versus percentage of damage on plants. Second instar: $F = 28.5864$, $P < 0.001$ (grey line); third instar: $F = 16.685$, $P < 0.001$ (black line); fifth instar: $F = 33.879$, $P < 0.001$ (dashed black line).

experienced with odors of plants fed on by second instars showed a slight but significant preference for the odor of plants fed on by third instars (Figure 8B). Interestingly, fewer females chose the odor of plants attacked by fifth instars, but this trend is likely due to the lower quantities of volatiles emitted by these plants (Figure 9), which showed less damage than plants eaten by second and third instar larvae.

DISCUSSION

In all cases, the three different instars induced an emission of volatiles in maize. Regurgitant of the three instars induced similar volatile blends, both in terms of quantity and quality. The only difference was a higher emission of phenethyl acetate and α-humulene (two of the minor compounds) when plants were incubated in fifth instar regurgitant (Figure 2). The composition of regurgitants of the three different instars tested showed no major differences (Figure 3), and all three instars produce the known elicitor volicitin (Alborn et al., 1997).

The total amount of volatiles released was correlated with the amount of damage done by the larvae. No differences were found among instars when comparing plants with equal amounts of damage. This was confirmed by mimicking

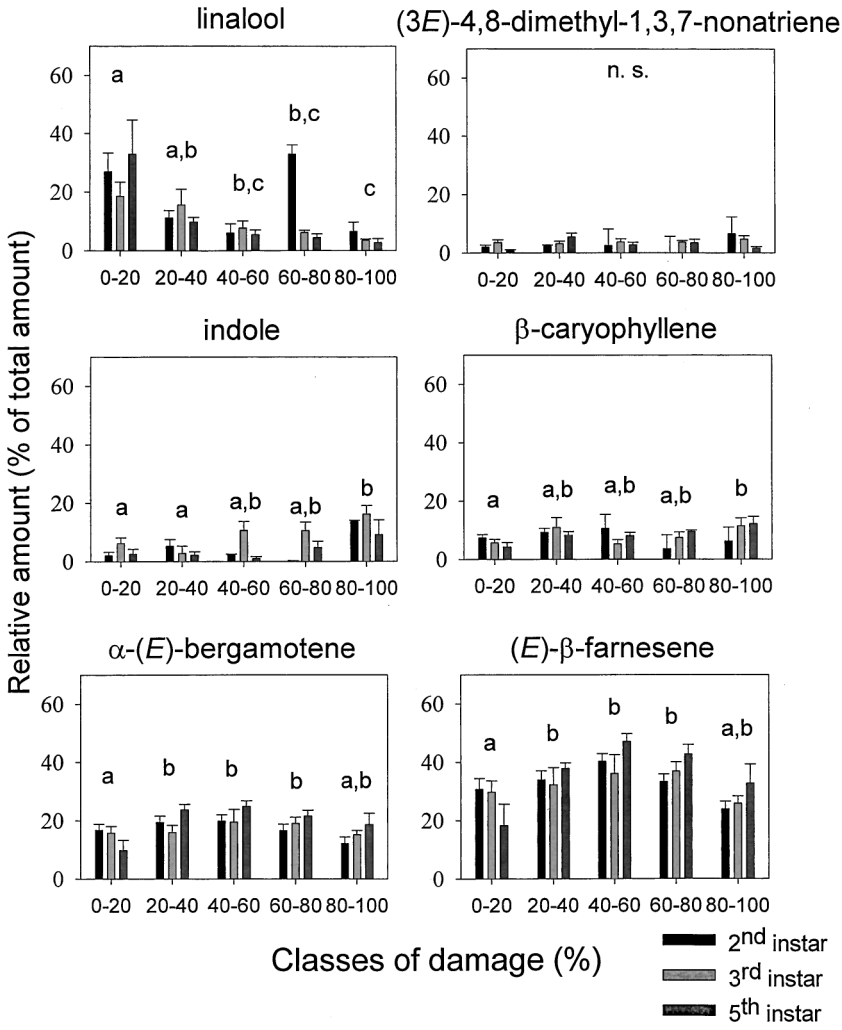

FIG. 5. Average relative amount of the six main induced volatiles (mean + SE) emitted for five classes of damage done on plants. Different letters above bars indicate significant differences between classes of damage ($\alpha = 0.05$) after Dunnett's T3 *post hoc* test.

the damage done by the different developmental stages. The type of damage did not affect the emission of induced volatiles. When larvae were feeding directly on maize plants, differences in the relative amounts of the six dominant compounds were due to the amount of damage inflicted on plants. Females of the parasitoid *M. rufiventris* did not show differences in their preference for odor induced by the

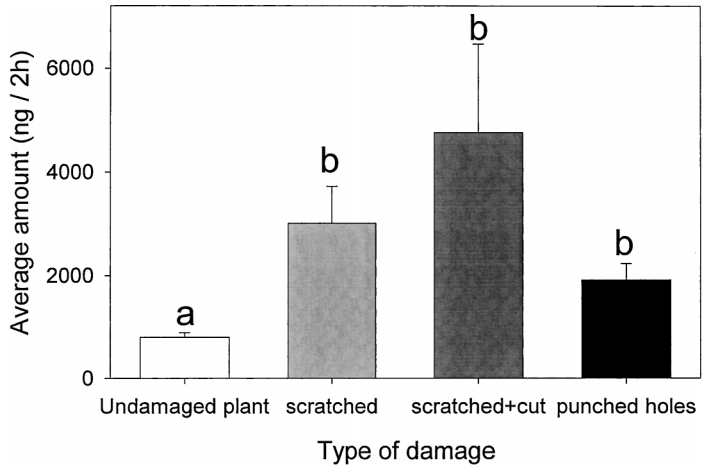

FIG. 6. Average total amount (mean + SE) of induced odor emitted by maize plants after three different types of damage. Undamaged plants served as controls. Different letters above each bar indicate significant differences after Student-Newman-Keuls *post hoc* test ($\alpha = 0.05$)

different instars. There may be some subtle differences in the odor profiles that we may not have detected because of the limitations of the analytical methods used, but the response of the wasps does not provide any evidence for differences (Figure 8).

In contrast to the results found here, Takabayashi et al. (1995) found that different developmental stages of *P. separata* differently affected the emission of volatiles in maize and that the parasitic wasp *C. kariyai* uses these differences to locate suitable young hosts. Late instars of *P. separata* do not induce the release of volatiles, while early instars do. Takabayashi et al. (1995) suggest that the plant releases different blends of induced odor that provide the parasitoid with information on stage and suitability of the herbivore. This hypothesis was not supported in a system studied by Mattiacci and Dicke (1995). The parasitoid *C. glomerata* does not discriminate among cabbage plants infested by different instars of *Pieris brassicae* even though it also can only attack young instars. Female wasps are more responsive when they have previously encountered a suitable host and they are able to learn the surrounding odor during such an encounter (Turlings et al., 1993b; Vet et al., 1995). McCall et al. (1993) showed that *Microplitis croceipes* increases its responsiveness after encountering its host, and the effectiveness of this learning process also increases with the number of experiences the wasps had previously. Turlings et al. (1993b) described associative learning in *C. marginiventris* and how this generalist is capable, after a single experience, to distinguish among

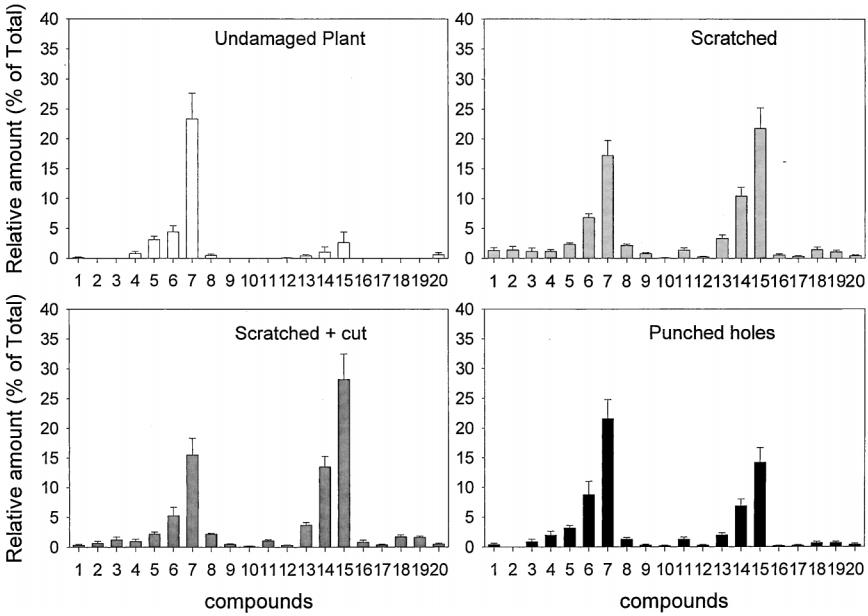

FIG. 7. Average relative amount of the different compounds of the induced odour blend (mean + SE). (1) (*Z*)-3-hexenal, (2) (*E*)-2-hexenal, (3) (*Z*)-3-hexen-1-ol, (4) (E)-2-hexen-1-ol, (5) β-myrcene, (6) (*Z*)-3-hexen-1-yl acetate, (7) linalool, (8) (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (9) phenethyl acetate, (10) indole, (11) geranyl acetate (12) unknown, (13) β-caryophyllene, (14) (*E*)-α-bergamotene, (15) (*E*)-β-farnesene, (16) α-humulene, (17) unknown sesquiterpene, (18) β-bisabolene + (*E*,*E*)-α-farnesene, (19) β-sesquiphellandrene, (20) (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

odors emitted by plants that are eaten by different host species. For *M. rufiventris*, even when female wasps had a previous experience with the odor of maize plants fed on by the different developmental stages, they did not distinguish among the induced odor of plants. The preference for plants attacked by third instars when females had an experience with the odor of maize fed on by second instars (Figure 8) also indicates that the difference among the blends is small. This can be explained by the fact that the amount of induced odor emitted in this experiment was higher for the maize plants attacked by third instars (Figure 9). The behavior of *M. rufiventris* confirmed the results obtained by analytical methods that induced maize odor does not significantly differ when different larval instars feed on the plant. Hence, the volatile signal appears limited in the specific information it provides.

The specificity of induced signal in plants has been demonstrated in several tritrophic systems. The aphid parasitoid *Aphidius ervi* can distinguish between plants fed on by its host *Acyrthosiphon pisum* and plants fed on by a nonhost

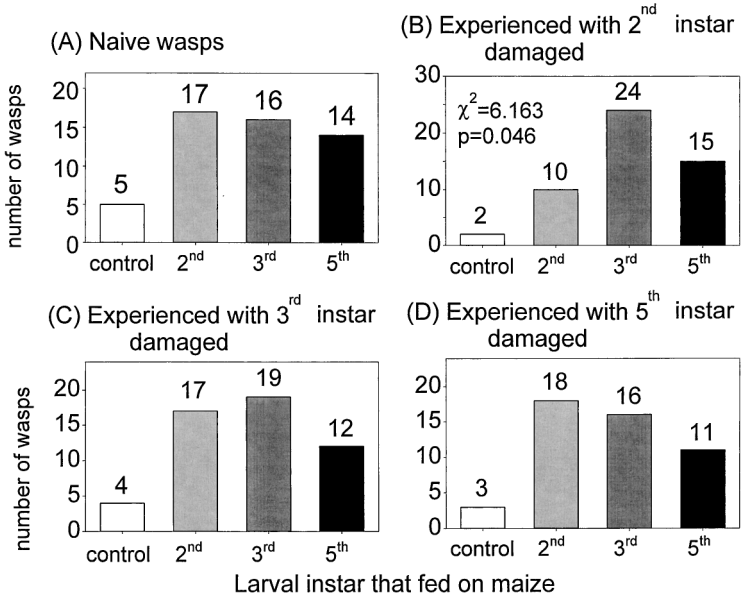

FIG. 8. The number of wasps that chose the odor of maize plants damaged by either second, third, or fifth instars, or undamaged plants. The female wasps were either naive or experienced by allowing them to oviposit in a second instar on a plant that had been damaged by either second, third, or fifth instars.

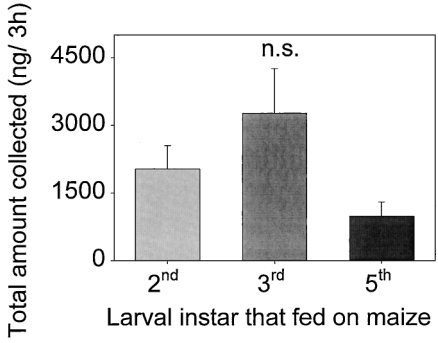

FIG. 9. Total amount of induced odor (mean + SE) released by plants fed on by second, third, and fifth instars during the 3hr of collection in the six arm olfactometer collection system. No significant differences were found among the quantity of volatiles emitted by the plants ($F = 2.793$, $P = 0.079$).

aphid. This distinction is suggested to be due to a specific compound, 6-methyl-5-hepten-2-one, of which the emission increases only when the parasitoid's host feeds on the plant (Du et al., 1998). De Moraes et al. (1998) found that the specialist parasitoid, *Cardiochiles nigriceps*, can use plant volatiles to differentiate among plants infested by its host from plants infested by a closely related non-host species. In this case, the ratios of induced volatiles in the odor blend from plants attacked by the two different noctuid hosts was somewhat different. Thus, in some systems, induced odors from plants seem to give parasitoids information on the suitability of the herbivore feeding on the plant, while in others (e.g., Mattiacci and Dicke, 1995) they do not.

Due to differences among plant species and genotypes (Gouinguené et al., 2001) as well as variation caused by abiotic factors (Gouinguené and Turlings, 2002), induced plant signals can be variable. It seems unlikely that generalist parasitoids such as *M. rufiventris* have adapted to respond to specific plant cues. Our results confirm this notion; the volatile signal induced by *S. littoralis*, a generalist herbivore, on maize does not seem to be a reliable indicator of herbivore stage. Other cues than induced plant odors might provide parasitoids with more specific information. These are likely to come from by-products of host larvae. *C. marginiventris*, for instance, is also attracted by herbivore frass and moth scale of *S. frugiperda* (Loke and Ashley, 1984). Such kairomones that come directly from the host are more reliable than plant odors, but far less detectable (Vet and Dicke, 1992). Considering the high variability in the readily available induced plants odors, even within a species (Gouinguené et al., 2001), it may be adaptive for the wasps to be flexible and exhibit specificity in their responses only after repeated experiences with rewarding odor blends.

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